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The Tocopherols

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VITAMIN E—
The Tocopherols

By HENRY A. MATTILL, Ph.D.*

Introduction

During the relatively few years since the tocopherols were recognized as members of the vitamin family they have revealed most interesting traits. Few of the vitamins have been associated, experimentally, with as many and varied physiologic processes as have the E vitamins. Because of their ubiquity in natural foods and the diverse manifestations of their lack in different species, the mechanism of their action has not been elucidated nor has their possible role in human nutrition been established.

The discovery of vitamin E as a distinct entity was made in several laboratories almost simultaneously in 1923-25 (1) as a result of the observation that for reproduction the white rat required a fat-soluble substance beyond the accessories already recognized as indispensable. The monumental monograph of Evans and Burr (2) described the nature of the reproductive failure and some of the chemical and physiological aspects of the new vitamin. Subsequently it was isolated by the Emersons and Evans (3) from the nonsaponifiable portion of wheat germ oil; they called it tocopherol, an alcohol concerned with the bearing of young.

Its chemical structure (see Fig. 1) was definitively formulated by Fernholz (4) as that of a methylated chromane ring containing the phytyl group as a substituent on carbon 2 and a hydroxyl group on carbon 6 para to the chromane oxygen. In the meantime, although uncertain whether the ring was chroman or coumaran, Karrer and his coworkers (5) had synthesized a physiologically active compound from trimethyl hydroquinone and phytyl bromide, thereby demonstrating a high degree of organic-chemical and biological prescience.

Many other investigators, by degradative and synthetic studies, made contributions to our present knowledge of the chemistry of tocopherol and its derivative, among them Johns in Göttingen, Todd and the lamented Drummond in London, Smith in Minnesota, Hickman and Harris in Rochester, and Mattill and his colleagues at Iowa. By the way of summary, there are several tocopherols, α being 5, 7, 8-trimethyl tocol (Karrer suggested

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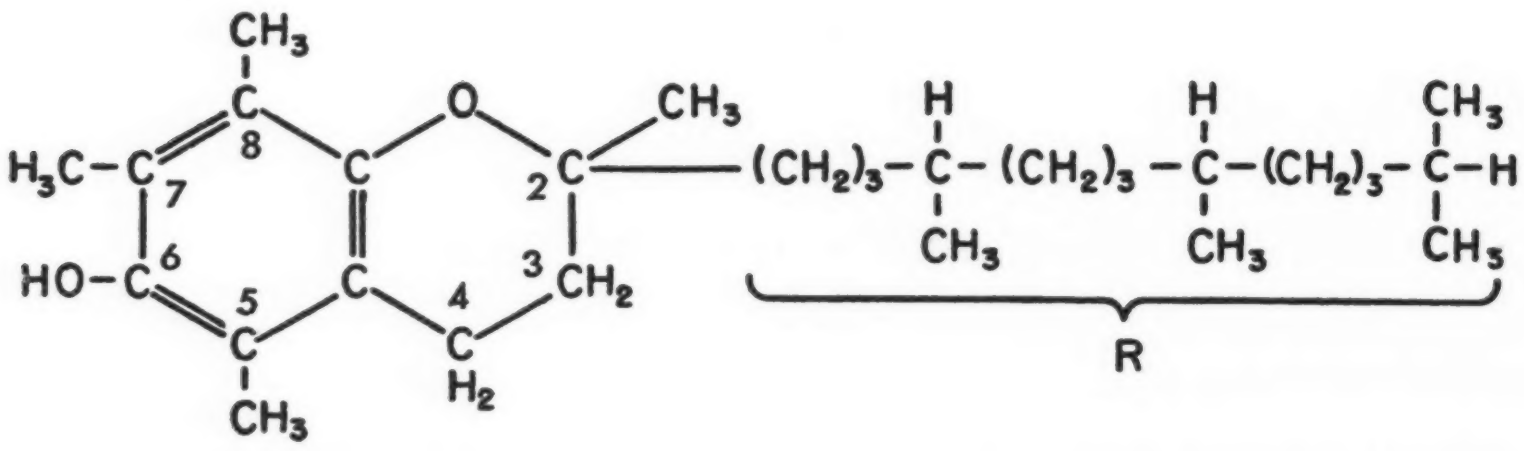
FIGURE 1.



***Alpha*-tocopherol:** $C_{29}H_{50}O_2$: Mol. wt. 430.69

that the unsubstituted tocopherol be called tocol), β and γ 5, 8- and 7, 8-dimethyl tocol, respectively; δ-tocopherol is 8-monomethyl tocol. The principal oxidation products are tocopheryl quinone (chromane ring opened) with an intermediate free radical and an epoxide, and a chromane-5, 6-quinone, sometimes known as the red quinone, in which the vulnerable methyl group on carbon 5 has been replaced by oxygen. All of these compounds are reddish yellow oils; some of them have been crystallized.

Assay

For quantitative determinations the amount of red quinone produced by the action of nitric acid (6) can be measured colorimetrically or spectrophotometrically, but wider use, both on macro and micro scales, has been made of the more specific ferrous dipyridyl color when ferric iron is reduced by tocopherol, which is thereby oxidized to tocopheryl quinone. The interference of carotene can be obviated in several ways. Chromatographic separation of the tocopherols on either alumina or paper provides a new method for separating the individual tocopherols in mixtures.

The relation between chemical constitution and physiological properties is always an engaging subject. The relative biological potency of the tocopherols, as measured percentually by the rat fertility test is approximately 100, 40, 8 and 1 for the α, β, γ and δ respectively. The hydroxyl group on carbon 6 is essential and the isoprene chain may not be shortened or lengthened without producing inactivation.

Because the proportions in which the different tocopherols occur in natural sources vary considerably, the tedious bio-assay with E-deficient rats is usually indicated. In the application of this procedure many variables must be taken into account. Of the various modifications that have been proposed, the technique of Mason (9) is perhaps the most precise, the criterion of fertility being the presence of at least two viable fetuses and four implanta-

tion sites in the uterus on the sixteenth day of pregnancy; the animal must have been reared, from before weaning, on an E-deficient diet, the test dose should be administered within five to ten days of a positive mating as indicated by cessation of estrus and by the placental sign (blood leak) which should appear about the twelfth day. For adequate validity there must be statistical treatment of the data, relating dosage to litter efficiency either on a standard curve or more simply by the method of probits (10).

The tocopherols are the principal antioxygenic substances (for fats) in natural foods (11). Indeed, this property of delaying the development of rancidity, vicariously, was the occasion of their original emergence: rats were sterile on diets containing lard or cod liver oil even if vitamin E was originally present and in contact long enough with the oxidizing fat. The in vitro antioxygenic activity of the four tocopherols is in the reverse order of their biological potency, a fact which prevented the early recognition of the identity of vitamin and antioxidant. Esterification with acetate, phosphate or succinate abolishes the antioxygenic property of the tocopherols; the esters are hydrolyzable in vivo and are relatively stable. The phosphate and succinate are soluble in water and the crystalline succinate has been suggested for use as a standard (12).

Vitamin E and Reproduction

The quite different effects of deprivation on the reproductive apparatus of male and female rats are typical of the difficulties encountered in searching for functional explanation of the action of tocopherol. In the male the damage to the testes, with complete loss of spermatogenesis, is irreversible. In the female the ovaries retain their normal function; the fault is in the embryo and its connections such that the fetus dies from lack of blood supply. The role of tocopherol in the male rat may be related to the metabolism of nuclear substance, whereas in the female it seems to be concerned with permeability. Both of these aspects are germane to an understanding of the signs of deficiency in other species and were earlier discussed comprehensively by Mason (13).

Like the rat, the female mouse requires vitamin E for reproduction, but the male mouse does not (17). Many other species may also require it for reproduction but in its absence they succumb to other disabilities before they attain maturity.

Vitamin E and Muscles

Aside from sub-normal growth and the inability to procreate, rats may reveal no outward signs of malnutrition until they have been on the deficient

diet for perhaps a year. They then begin to show a slowly increasing lameness which finally leads to an inability to obtain food, and the animals end their days in complete helplessness (14). This chronic paralysis is different from the acute paralysis first observed in many of the young from borderline E-deficient dams during the few days just prior to their weaning (15). This paralysis begins suddenly in the hind legs, may be complete in twenty-four hours and the animals appear to die of inanition. The primary lesion is a degeneration of muscle (16) which is reversible if vitamin E is administered early enough, in the case of older rats before necrosis is too extensive. For reasons yet unknown, nursling rats may show spontaneous recovery, partial or complete, and appear to be normal until they mature and are found to be infertile.

Most remarkable is the nutritional muscular dystrophy (18) which can be produced in adolescent rabbits within as short a time as three weeks when they are placed on an E-deficient diet. It resembles the acute dystrophy of nursling rats. The first easily observable sign is an increase in urinary creatine, which must be carefully distinguished from that produced by the slight inanition attending the shift to the experimental diet. Soon there is a gradual loss of muscle tone, failure to retract the hind legs when they are extended, followed by inability of the animals to right themselves and complete prostration, with amazingly rapid loss in weight. Timely administration of tocopherol (19) may rescue the animals from their apparently painless fate, the duration of their reprieve depending on the amount of tocopherol given.

Meantime marked changes take place in the skeletal muscles (20). Chemically there is a large loss in creatine, even to half the normal figure, as well as decreased nitrogen and phosphorus and creatine phosphate, and an increase in sodium chloride and in nucleic acids (21). Histologically there is initially some degeneration of the sarcolemma and slight loss of cross striations which later almost disappear and there is replacement tissue with many giant cells and polymorphonuclear leucocytes. Histologically there is hyalinization, loss of cross striations and coagulative necrosis of many muscle fibers followed by connective tissue replacement and by considerable regeneration of new fibers. It is highly significant that these observable lesions appear subsequent to some of the chemical changes, especially as regards the loss of creatine; the biochemical fault comes first (22).

Profound abnormality of muscle can be similarly produced in other laboratory animals, such as guinea pig, mouse, hamster, dog and monkey and in the duckling. "Stiff lamb disease" and the white muscle disease of calves dependent on milk from cows receiving inadequate vitamin E can be cured by administering tocopherol.

A striking characteristic of dystrophic muscle of laboratory animals is its heightened oxygen consumption in vitro. Superficially this suggests an anti-oxygenic action of the tocopherols but the relationships are by no means so simple. Certain enzyme systems prepared from muscle have been inhibited by the presence of the water-soluble esters of tocopherol, such as phosphate or succinate, but it is becoming increasingly clear that this may be due to the non-specific detergent activity of these unnatural compounds. These, and most other aspects of the physiology of the tocopherols were exhaustively presented at the vitamin E symposium in New York in 1949 (23).

Since the so-called energy rich phosphate bonds are a means of conserving energy, in muscle particularly as creatine phosphate, it is tempting to conclude that the capacity to form them is diminished when vitamin E is absent. The energy is wasted instead of being stored. This remains to be proved; the addition of tocopherol itself to a Warburg flask has never altered the course of events.

It is possible that the altered metabolism in degenerating muscle tissue is not primarily of carbohydrate; the R.Q. does not differ from that of normal muscle preparations. The involvement of protein is suggested by the great outpouring of creatine, by the influence of a low-protein diet in hastening the onset of dystrophy by the increased output of allantoin, and the diminished activity of certain enzymes involved in amino acid changes.

Cardiac muscle of E-deficient rabbits also has a high oxygen uptake in vitro and histologically shows similar damage especially in the papillary muscles, ventricular walls and septum (24). Electrocardiograms confirm the severity of the lesions.

Pigmentation

Accompanying these degenerative processes a curious and very inert pigment appears in various tissues, (25), recognizable by staining characteristics and by its fluorescence. It was first observed as a brownish discoloration of the uterus (26) and it commonly also occurs in adipose tissue where it is often accompanied by fat peroxides (27). It may thus represent unusable polymerized products of unsaturated fatty acids produced by peroxidation in the absence of a necessary fat stabilizing agent or it may have its origin in certain amino acids, notably tryptophan. The opposite condition, depigmentation, is produced in the maxillary incisors of rats on high fat diets lacking in vitamin E.

Chicks

Chicks have provided some interesting observations because the vitamin

E content of the hens' food, and of her eggs, can be correlated with hatchability, and with the viability of the chicks. With cod liver oil in the vitamin E-deficient feed, hatchability is low and embryonic mortality is high, because of interruption of the vitelline circulation. When newly hatched normal chicks are reared on vitamin E-deficient diets, they may exhibit either of two disorders, exudative diathesis or encephalomalacia. The former is the result of increased capillary permeability (28); to produce it the diet must contain more than traces of fat and also fresh or partly rancid cod liver oil. The peroxide content of very rancid fats is low.

Encephalomalacia, first recognized by Pappenheimer and Goettsch as of nutritional origin (29), is characterized by sudden ataxia, spasms and prostration. Cerebella degeneration is grossly visible. A similar disorder occurs under field conditions "crazy chick disease," and the monograph (30) which traces the protective factor through the vegetable oils, ultimately to tocopherol, is an informing document.

Many factors other than vitamin E modify the experimental results with chicks on E-deficient diets. Thus, a diet which produces diathesis can be altered so as to produce encephalomalacia by increasing the content of lard and protein and decreasing the carbohydrate (31). The severity of both disorders is lessened by including in the diet certain reducing substances, notably methylene blue, and since it was also recently claimed that this dye can partially replace tocopherol in preventing the sterility of female rats on tocopherol-deficient diets (32), the case for tocopherol as a biological antioxidant is strengthened, depending on the interpretation.

In a series of annual conferences dealing with biological antioxidants (33) the physical, chemical and biological implications of the subject were exhaustively presented. If living organisms are constantly exposed to the damaging effects of active peroxides, whether produced by rancidifying fats or by ionizing radiations, and whatever the results of their noxious action, some palliative or detoxicating agents are demanded if life is to continue. Various water-soluble substances are known to act in this manner and how important the fat-soluble tocopherols may be, and how they fulfill their mission, if such it is, remain to be explored.

Miscellaneous

Certain further observations on the effects of vitamin E deprivation must be sketched into the picture, although just where they belong it is difficult to say.

Massive necrosis of the liver is produced in young rats on a low protein E-deficient diet or one containing oxidized casein; some protection is afforded

by increasing the casein content or by administering tocopherol (34). The necrogenic properties of some yeasts in contrast to the innocuous character of others is concerned with the presence, in the latter, of a powerful protective factor which is not cystine or vitamin E (35). Gastric ulcers produced in adult rats by a low protein diet or by fasting are favorably influenced by tocopherol if the diet contains unsaturated fats (36).

Administration of alloxan to rats produces not only diabetes but may also cause hemolysis of the red blood cells. With tocopherol in the diet, the latter but not the former is obviated (37). The blood cells of animals deficient in tocopherol are hemolyzed in vitro by the oxidation products of alloxan; this hemolysis is prevented by the addition of tocopherol (38). The cells of tocopherol-treated animals are not affected.

Much attention has been given to the possible relation of tocopherol to the action of certain glands with internal secretion and to malignant processes but the information is very circumstantial and the variables are too numerous to support definite conclusions.

Sources

The richest sources of the tocopherols include green plant tissue (where it is usually accompanied by carotene) legumes, eggs, liver and most vegetable oils and the products containing them such as cereals and margarine; with the exception of durum wheat, degerminated seeds contain little. In milk the amount seems to depend directly on the cows' intake whether as the result of natural seasonal supply or supplementary feeding (39); there is more in colostrum than in the later milk.

In all of these sources the tocopherols occur free and not as esters, but there is some evidence for the formation of conjugates with native proteins such as lipid-free plasma albumin, in which a stable opalescent solution can be made (40). Skeletal muscles contain more E than liver tissue but the latter is the chief repository in the rat when the intake is high (41). There is also evidence that body fats can be similarly enriched, thus increasing their stability on storage (42). There can be no doubt of the sparing action of tocopherol on vitamin A (43); account has been taken of this in the specification that the basal diet for vitamin A assays should contain vitamin E, whatever other possible stabilizers may be present.

Requirements

In view of the many variations as between species and even within species it is obviously not possible to state specific requirements with any degree of

accuracy unless the specifications of the diet and the conditions of the functional measurement are carefully indicated. Thus the median fertility dose (M.F.D.), 0.75 mg. of α-tocopherol, for the female rat, holds only for the particular conditions of the test. To prevent paralysis in nursling rats it is necessary to progressively increase the dose if the administration of vitamin E is delayed as between the tenth and seventeenth day. The prevention of dystrophy in the rabbit has been estimated to require 0.2-0.4 mg. daily; the minimum need of the laying hen is 1.2 mg. per day.

Whether domestic animals on their natural feeds require supplements of vitamin E for best returns in growth and in reproduction is a moot question. Few if any sufficiently controlled experiments have been made. More than twenty years ago Vogt-Moller (44) reported the successful treatment of sterile cows with wheat germ oil and this contention has been supported and denied. Indeed, the need of vitamin E for reproduction in domestic animals cannot be said to have been clearly demonstrated. The results of recent experiments on swine are more indicative of a requirement for vitamin E (42, 45) in reproduction.

The requirement of the human organism will likely never be determinable directly. If the function of vitamin E may be quantitatively related to the 0.73 power of the body weight the daily requirement of an adult person might be about 30 mg. (46). Surveys here and abroad have indicated an average intake of 6 mg. or less (47). It must be emphasized that variations in the kind of tocopherol present, the presence of rancid fat, and numerous other factors such as the availability and absorbability would influence the requirement for vitamin E.

Clinical Considerations

Based on experimental observations on cardiac muscle in vitamin E-deprived animals the value of vitamin E therapy in certain heart conditions has been zealously extolled. The need is for more, and more carefully controlled, clinical observations. The same is true as regards the treatment of human sterility.

The conquest of some of the muscular disorders found in man has a more hopeful prospect. The administration of tocopherol has not effected cures but certain of the clinical symptoms, such as creatinuria, have been partially abolished. Since tocopherylquinone is as effective as tocopherol in the cure of experimental dystrophy in the rabbit (48), it has been suggested that the former may be the active principle, and that the dystrophic patient lacks a mechanism for the proper conversion; the fault is a metabolic one. There is

reasonable hope that as the chemical pattern of muscle function is unravelled, the role of tocopherol may become clear.

Summary

That vitamin E is an important and essential substance for optimum nutrition is evidenced by the serious and varied results which follow a deficit of this vitamin for many species. Although extensive experimentation has been directed toward the elucidation of the specific biochemical role of vitamin E, the exact nature of this function remains obscure. No clearly defined need for vitamin E has been demonstrated for the human. This may be but a reflection of numerous research difficulties and lack of sufficient knowledge.

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EDITORIAL NOTE

The present number completes Volume 13 of *Borden's Review of Nutrition Research.* From its inception, this review has attempted at all times to present sound discussions of the important subjects in the field of nutrition research. An examination of the index of the first 13 volumes is almost like reading a map or following the trail of the highlights in nutrition research during a most rapidly changing era. Because the *Review* has remained a completely non-commercial effort designed to serve the public interest by spreading knowledge about the science of nutrition, it enjoys the respect and acceptance of the professional people concerned with nutrition work.

The *Review* has an audience of about 15,000 readers — physicians, dietitians, public health workers, nutritionists and scientists in numerous academic centers, etc. Because of this extremely varied audience, it is a constant challenge to provide material that has uniform appeal. Every effort has been made to develop as high a degree of "readability" as possible.

The rapid developments and expanded knowledge of nutrition have led quite naturally to some degree of complexity and to specialization by workers in the field. In an effort to present these rather finite areas of nutrition, the *Review* has decided to turn to numerous individuals for preparation of the topics, primarily with the idea of bringing varied points of view to bear on the problems of interest.

In further consideration of the above, it has been decided that an even more effective presentation of carefully selected topics can be provided on the basis of six publications per year instead of the customary ten.

This will allow the further advantage of making available more than the customary space for a topic of special interest. This plan will be put in effect starting with Volume XIV.

The editorial board wishes to thank all of the readers of the *Review* for their continued interest and patience, and invites any criticisms and suggestions which would lead to improvements in *Borden's Review of Nutrition Research.*

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